THE EARLY DEVELOPMENT OF THE HYPOPHYSIS IN CHELONIA.

CHARLES ZELENY.

THE following observations on the early development of the hypophysis in Chelonia are offered at this time because they throw positive light upon the derivation of the organ concerning which we at present have several conflicting views. The paper is based upon sections of Aspidonectes spinifer Ag., Chelydra serpentina L., and Chrysemys marginata Ag., which show that in these forms the hypophysis is undoubtedly of epiblastic origin. Incidentally some points regarding the relation of the preoral gut to the notochord and the head cavities will be noted, but this subject will receive fuller treatment in a subsequent paper. For the sake of convenience the subject matter will be considered under the following heads :

1. A general outline of the literature dealing with the early development of the hypophysis among vertebrates.
2. Material and methods of preparation.
3. Description of stages.
4. Summary and conclusion.

Literature.

It would be out of place in a paper such as the present to give any detailed account of the views which have been held regarding the subject under discussion. A bare mention of a few of the upholders of each of the principal views, giving the group upon which work was done, must suffice.

1. Those who have claimed a hypoblastic origin for the hypophysis are Luschka (**'69**), Mammalia ; W. Müller (**'71**), Vertebrata in general ; Dohrn (**'82**), Teleostii, later extended to Vertebrata in general ; Hoffmann (**'88**), Lacerta ; Prather (**'99**), Amia.

2. Those who have claimed an epiblastic origin for the hypophysis are Mihalkovics ('**77**), Aves and Mammalia; Balfour ('**78**), Elasmobranchii; Orr ('**87**), Lacerta; Lundsborg ('**94**), Salmonidae; Dean ('**96**), Amia; Haller ('**96**), Vertebrata in general; Hoffmann ('**96**), Elasmobranchii; Melchers ('**99**), Lacertilia. Of these Orr ('**87**), although he describes the hypophysis as of epiblastic origin and so figures it in his sections, nevertheless considers it probable that hypoblast cells may take some part in its development.

3. Those who have claimed that the hypophysis is partly of epiblastic and partly of hypoblastic origin are Kupffer ('**93**), Acipenser and Ammocoetes; Valenti ('**95**), Amphibia (Bufo); Nussbaum ('**96**), Mammalia; and considered probable by Orr ('**87**), Lacerta.

It is of special interest to note that even this partial list gives each of the three views a large number of the groups of vertebrates upon which to base its general character.

Material and Methods.

The material upon which the following observations are based was obtained in Minnesota during the summers of 1898 and 1899. The embryos of Aspidonectes are from Grey Cloud Island in the Mississippi River below St. Paul, and those of Chrysemys and Chelydra are from the neighborhood of Hutchinson. The fixing fluid used was Gilson's mercuro-nitric mixture. The embryos were stained *in toto* in haemacalcium or in paracarmine. The series of sagittal sections were found to be the most helpful in determining the cell-layer from which the hypophysis is derived, and the following descriptions are taken entirely from such sections.

Description of Stages.

The following stages will be figured and described:

Stage A. The hypophysial evagination has not yet appeared.

Stage B. The hypophysial evagination is very evident and the pharyngeal membrane has not yet been broken.

Stage C. Slightly older than Stage B. The pharyngeal membrane has been broken.

Stage D. The downward growth and enlargement of the fore-brain have pushed the hypophysis back from its primary relation to the broken ends of the pharyngeal membrane.

Stage E. The hypophysis has been shifted backward so as to assume a position relatively far back in the pharyngeal cavity.

Stage F. The hypophysis has become differentiated into a terminal, broad, sac-like part and a narrower connecting stalk.

Stage A.

In an embryo with five or six mesoblastic somites and in which the medullary folds have not yet united above to enclose a medullary canal the hypophysial evagination has not begun to form. A median sagittal section of such an embryo, however, shows the relation of the parts surrounding the point at which the hypophysis will appear at a later stage. Fig. 1, Pl. I, represents a diagrammatic median sagittal section of an embryo of Chrysemys marginata 2.5 mm. in length and in which there are five distinct mesoblastic somites with indistinct traces of a sixth. The figure is a combination of the six sections nearest to the median line. Although the medullary groove is still open above, the medullary folds in the head region have grown to a considerable height, as represented by the dotted line *D.* At the same time the whole anterior region has been folded and bent downward. The fold of the blastoderm which comes up over the head as a result is the proamnion (*Pa.*), and consists of epiblast and hypoblast. At the bottom of the head fold the hypoblast has traveled back much farther than the epiblast, leaving a space in which the cardiac mesoblast (*Cm.*) develops. As we trace this hypoblast (*End.*1) forward along the floor of the fore-gut (*F.G.*) we find that it is bent ventrally so as to come into contact with the epiblast. This point of contact, represented by the double-headed arrow ($\updownarrow$), is the region at which the mouth will be formed. In front of this we may recognize a short, wide, preoral gut (*Pr.G.*). This is a

true preoral gut and not an apparent one caused by the downward bending of the head region; for we must consider the sharp angle in the hypoblast at *Pr.G.* in the figure, and not the part back of it where the mouth will appear, to have been the original extreme anterior end. Thus we may consider all the hypoblast below and posterior to this angle to belong to the ventral wall of the alimentary canal, and all that above and posterior to belong to the dorsal wall. Following the dorsal wall we find that it immediately divides into two parts, one of which is the notochord (*Nc.*) and the other the dorsal wall of the gut (*End.2*). For this reason the hypoblast forming the ventral wall of the alimentary canal, and continued out at the ends of the embryo into the flat outlying blastodermic region, may be called the "primary hypoblast," and the hypoblast of the dorsal wall the "secondary hypoblast." Rex ('97), in his work on the duck, and Davidoff ('99), in the embryo of Platydactylus, have found similar relations of the notochord to the hypoblast. The terms "primary hypoblast" and "secondary hypoblast," which are used above, are taken from the paper of the latter author. It is important to note in this connection that my sections show a distinct line of demarcation between the epiblast and the hypoblast in this region, so that the mass of cells surrounding the preoral gut is distinctly hypoblastic and not a mass of undifferentiated cells. The arrow with a feathered shaft ( ) shown in Fig. 1 in the epiblast directly under this point marks the position and direction of the future hypophysial pocket. The very plain line of division between the epiblast and hypoblast excludes the possibility that any of the hypoblast cells may take part in this ingrowth of the epiblast.

Stage B.

In an embryo 5.2 mm. in length and with twenty-one mesoblastic somites, such as is shown in median sagittal section in Pl. I, Fig. 2, and Pl. II, Fig. 3, the brain has developed with great rapidity. The cephalic flexure having proceeded at the same time, the region around the preoral gut is greatly compressed. The cavity of the preoral gut itself has become very

small and its dorsal wall is doubled back on itself. The notochord also at its anterior end near the point where it joins the hypoblast has become very much twisted and curved, giving it a knotted appearance in sagittal section. However, the parts are easily recognized as having the same relation as those in Stage A (Fig. 1). In Stage B (Fig. 2), as before, the dorsal wall of the fore-gut is the "secondary hypoblast" and the ventral wall the "primary hypoblast," the division line between the two being the point where the notochord joins the hypoblast. The pharyngeal membrane is still intact but breaks up very soon after this stage. Directly in front of the place where the mouth opening will appear the epiblast bends sharply back on itself to follow the brain, forming a pocket in which the cells are taller than in the neighboring parts of the epiblast. This is the beginning of the outpocketing which will eventually form the hypophysis. The evaginating layer of cells is clearly distinct from the hypoblast cells of the preoral gut, as is well shown in Pl. II, Fig. 3, which gives an enlarged view of the hypophysial region of Fig. 2. Starting from the condition of the stomodeal epiblast in the earlier stage as shown in Fig. 1, we see that an epiblastic groove was originally formed at the point marked by the feathered arrow (↖) just in front of the future mouth by the forward and downward bending of the fore-brain. It is at the bottom and in the middle of this groove that the thickening and outpocketing of the cells start, and later form the epiblastic pouch which becomes the oral portion of the hypophysis.

Stage C.

In an embryo but slightly older than the one last described the pharyngeal membrane has already been broken. Fig. 4, Pl. III, represents a diagram of a median sagittal section of such an embryo. On account of a lateral bend in the neck region it was not possible to obtain a section which would show the connection of the notochord and the preoral gut at the same time with the mouth opening and the hypophysial evagination. The canal (*H.C.*) between the two premandibular

head cavities is, however, shown, and the small mass of cells connected with it and directed toward the hypoblast of the fore-gut is the strand which connects the head cavities with the preoral gut. The preoral gut itself is not shown in this figure, the cavity (*F.G.'*) being a part of the fore-gut which has assumed a position anterior to the mouth because of the bending of the alimentary canal, which takes place at the same time with the cephalic flexure. Except for the break in the pharyngeal membrane the relation of the hypophysis to the epiblast is the same as in Stage B. The hypophysial outpocketing is here, as before, on the epiblastic side of the mouth opening and is undoubtedly made up entirely of epiblast cells.

Stage D.

In Fig. 5, Pl. III, we have a median sagittal section of a somewhat later stage. The points p and p' show the position of the ends of the broken pharyngeal membrane, and the dotted line (*P.M.*) between them represents the former position of the now ruptured membrane. The hypophysial pouch (*Hyp.*) is shown very distinctly on the epiblastic side of the membrane. It has, however, been pushed back from its original position with relation to the point p' by the rapid growth of the fore-brain. The anterior end of the notochord (*N.*) is still more curved and wrinkled than in the last stage, but it retains its connection with the anterior wall of the preoral gut (*P.G.*) by means of a string of cells. In this string of cells we see the section of the canal (*H.C.*) which connects the two anterior or premandibular head cavities. The hypophysial evagination from the very beginning is in close contact with the wall of the infundibulum, but the two layers of cells always remain clearly distinct. The epiblast cells of the hypophysis also remain clearly distinct from the hypoblast cells of the preoral gut.

Stage E.

Fig. 6, Pl. IV, is a diagram of a section of Chelydra serpentina. It shows a continuation of the same process of enlargement of the fore-brain and the consequent pushing

back of the hypophysial evagination, so that the latter finally appears to lie far back in the pharyngeal cavity. However, here, as before, the dotted line *pp'* shows the original position of the now broken membrane. At this stage the notochord has severed its connection with the fore-gut, but it is still joined to the mass of cells which connects the two head cavities. These cells still surround a canal, so that there is a passageway from the premandibular cavity on one side of the head to the corresponding cavity on the other side.

Stage F.

Fig. 7, Pl. IV, represents a median sagittal section of Aspidonectes spinifer, and Figs. 8 and 9, Pl. V, represent sections of the same series, respectively, three and six sections to the side of the median line. In these it is seen that there is no evidence of the canal which connected the two head cavities, and the notochord shows no sign of the former union with the hypoblast. The hypophysis has begun to constrict in the basal region and to enlarge in the terminal region so as to show a division into a narrower basal stalk and a wider terminal sac-like portion.

At a considerably later stage than the above the infundibulum sends out a pouch-like evagination, which from the beginning is in close contact with the wall of the oral sac and forms the infundibular part of the hypophysis.

Summary and Conclusion.

The foregoing series of sections furnish a clear chain of evidence in favor of the epiblastic origin of the hypophysis in Chelonia. Stage B (Figs. 2 and 3) itself is a conclusive proof of such an origin. Here with the pharyngeal membrane yet unbroken we find that the evagination is on the epiblastic side of the membrane. The distinct limiting line which marks the inner border of the hypophysial pouch excludes the supposition that the hypoblast cells may take a part in its formation. Even at the early stage represented in Fig. 1 there is

a distinct limiting line between the epiblast and hypoblast at the point where the hypophysis will later appear.

After the mouth opening has appeared (Stage C, Fig. 4) we find the hypophysis at first in the same relative position as in Stage B. Then the great increase in size of the fore-brain forces the epiblastic pocket to a position far back in the pharyngeal cavity, so that Stages D and E when considered alone would lead one to believe that the hypophysis is of hypoblastic origin in this group. That such is not the case is made evident by following the whole series of changes through all the different stages. There can be no doubt that in the Chelonia at least the oral evagination which goes to form the hypophysis is of epiblastic origin. As regards the infundibular portion there is no essential difference of opinion and its development need not be touched on here.

The bearing of the above conclusions on the paleostome theory of Kupffer and the neostome theory of Dohrn is of some interest. According to Kupffer, the hypophysis was originally a canal connecting the fore-gut with the epiblast, and represents an ancestral œsophagus which came up in front of the fore-brain and was replaced by the modern œsophagus at the time when the mouth was forced to a more ventral position by the enlargement of the brain. Dohrn, on the other hand, has picked out the epiphysis and hypophysis as remnants of the old annelid œsophagus which went up through the brain. Both of the above views presuppose some connection of the hypophysis with the hypoblast. The sections of turtle embryos which are described in the present paper give no evidence of such a connection at any stage.

In conclusion I wish to express my sincere thanks to Prof. H. F. Nachtrieb, who suggested to me the investigation of chelonian development and has aided me in many ways during the progress of the work.

DEPARTMENT OF ANIMAL BIOLOGY,
UNIVERSITY OF MINNESOTA, December, 1900.

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ABBREVIATIONS USED IN CONNECTION WITH THE FIGURES.

Ao.	Aortic arch.
Cm.	Cardiac mesoderm.
Ect.	Epiblast.
$End._1$	"Primary hypoblast."
$End._2$	"Secondary hypoblast."
Ep.	Epiphysis.
F. B.	Fore-brain.
F. G.	Fore-gut.
H. B.	Hind-brain.
H. C.	Canal connecting the two pre-mandibular head cavities.
Ht.	Heart.
Hyp.	Hypophysis.
Hyp. S.	Stalk of hypophysis.
I.	Infundibulum.
M.	Mouth opening.
M. B.	Mid brain.
Md.	Floor of medullary groove.
Nc. N.	Notochord.
Pa.	Proamnion.
P. G.	Preoral gut.
P. M.	Pharyngeal membrane.
. . .	Dotted line above *Md.* in Fig. 1 represents the height to which the medullary folds have risen.
↖	Position and direction of the hypophysial evagination.
↕	Pharyngeal membrane and position of future mouth opening.

PLATE I.

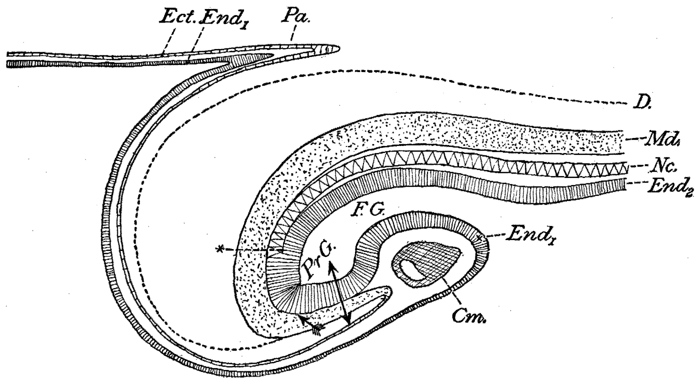

FIG. 1 (Pl. I), Stage A. — Combination diagram formed from the six sections nearest to the median sagittal section. Embryo of Chrysemys marginata. Mesoblastic somites = 5½. Lg. of embryo = 2.5 mm. × 62.

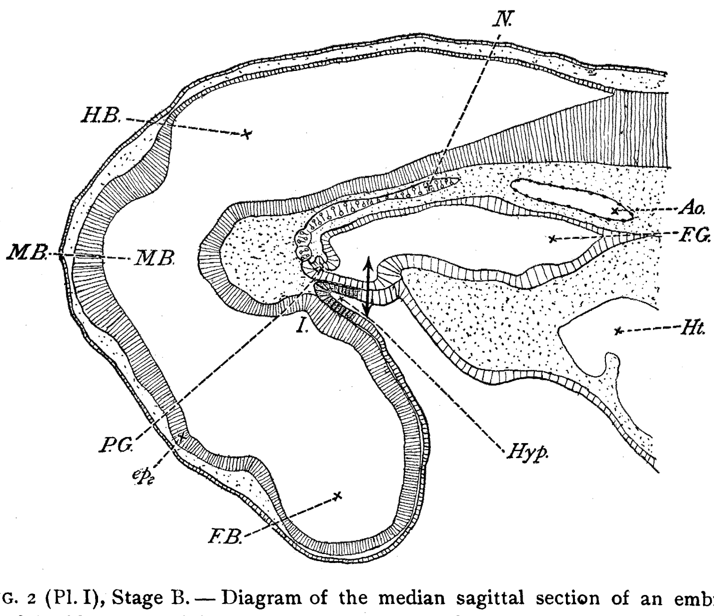

FIG. 2 (Pl. I), Stage B. — Diagram of the median sagittal section of an embryo of Aspidonectes spinifer. Lg. = 5.2 mm. Mesoblastic somites = 21. × 50.

PLATE II.

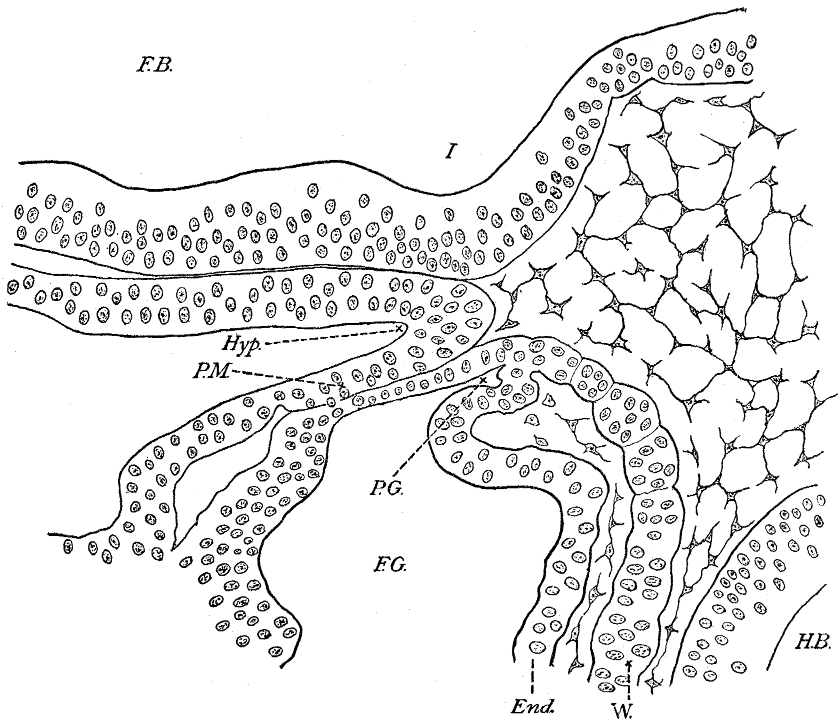

FIG. 3 (Pl. II), Stage B. — Median sagittal section of Aspidonectes spinifer. Part of Fig. 2, but with Magn. × 200.

PLATE III.

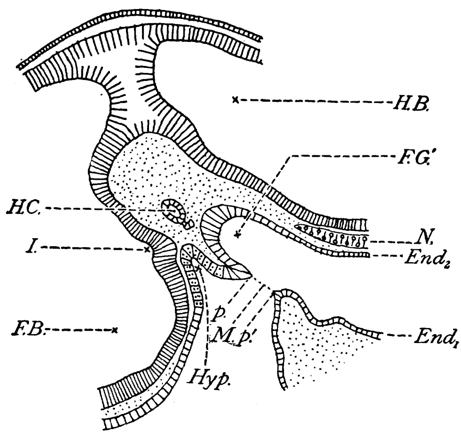

FIG. 4 (Pl. III), Stage C. — Median sagittal section of A. spinifer. Lg. = 5.7 mm. Mesoblastic somites = 21. × 50.

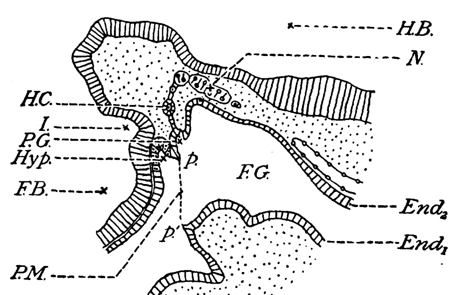

FIG. 5 (Pl. III), Stage D. — Median sagittal section of A. spinifer. Lg. = 6.0 mm. × 50.

PLATE IV.

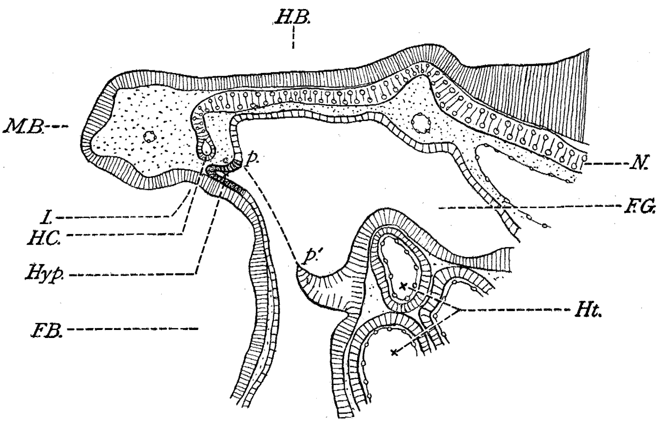

FIG. 6 (Pl. IV), Stage E. — Median sagittal section of Chelydra serpentina. Lg. = 7 mm. × 50.

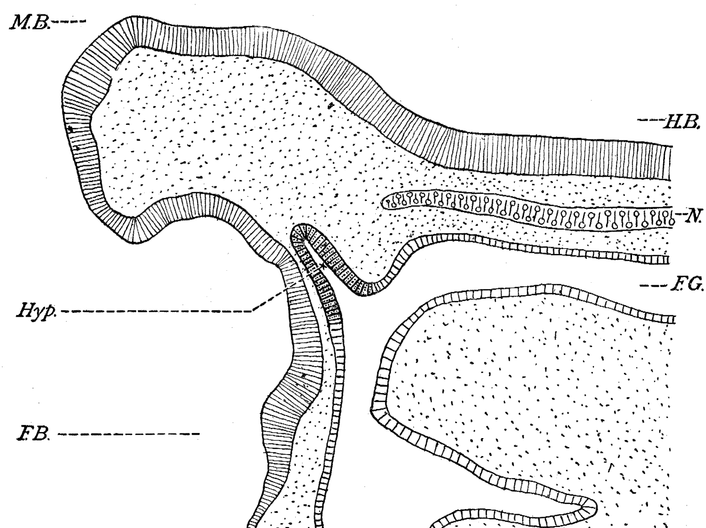

FIG. 7 (Pl. IV), Stage F. — Median sagittal section of A. spinifer. Lg. = 7.5 mm. × 50.

PLATE V.

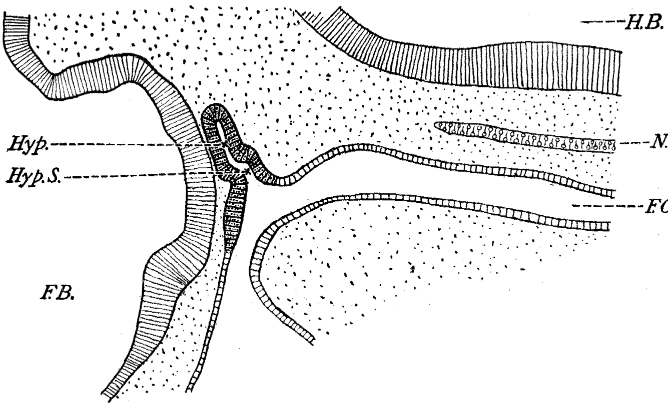

FIG. 8 (Pl. V), Stage F. — Sagittal section from the same series as Fig. 7, but three sections (40 μ) to the side of the median line. $\times$ 50.

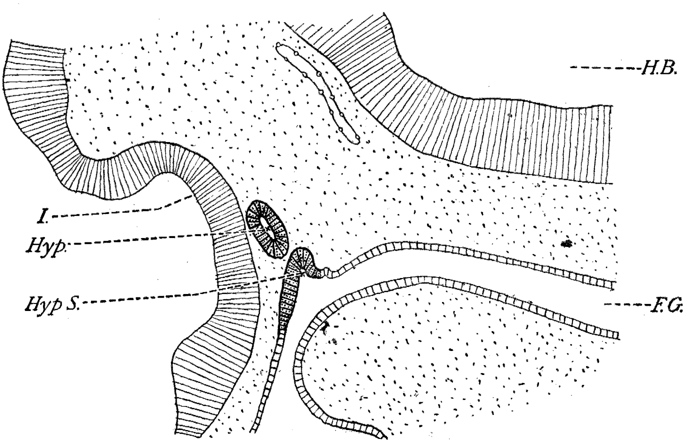

FIG. 9 (Pl. V), Stage F. — Sagittal section from the same series as Fig. 7, but six sections (80 μ) to the side of the median line. $\times$ 50.